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(54) Title: THE TREATMENT OF HIV AND OTHER VIRAL INFECTIONS USING COMBINATORY THERAPY

(57) Abstract

Novel antiviral combinations for the treatment or prevention of viral infections, in particular, HIV, are disclosed. This new antiviral therapy employs either DP-178 or DP-107, viral fusion inhibitors, in combination with at least one other antiviral therapeutic agent. The combinations of the invention are better than single therapies alone, and in certain cases are synergistic. The use of DP-178 or DP-107 is an ideal therapy to combine with another antiviral, given both the novel mechanism which this therapeutic blocks HIV transmission and the non-toxicity of the therapeutic.

THE TREATMENT OF HIV AND OTHER VIRAL INFECTIONS USING COMBINATORIAL THERAPY

1. FIELD OF THE INVENTION

This is a continuation-in-part of Serial No. 08/481,957, filed June 7, 1995.

The present invention relates to methods of treating viral infections, particularly HIV infection, using novel combinational therapy. The novel combinational therapy employs either the peptide DP-178, DP-107 or fragments, analogs and/or homologs thereof, and at least one other therapeutic agent.

DP-178 is a peptide corresponding to amino acids 638 to 673 of the HIV-1_{LAI} transmembrane protein (TM) gp41. DP-178 includes portions, analogs, and homologs of DP-178, all of which exhibit antiviral activity. Antiviral activity includes, but is not limited to, the inhibition of HIV transmission to uninfected CD-4+ cells. Further, the invention relates to the use of DP-178 and DP-178 fragments and/or analogs or homologs as inhibitors of retroviral transmission, in particular HIV, to uninfected cells, in both humans and non-humans. The present invention also relates to the antiviral peptide DP-107, a peptide corresponding to amino acids 558 to 595 of the HIV-1_{LAI} transmembrane protein (TM) gp41, that are present in other enveloped viruses. More specifically, the invention is directed to the use of DP-107, fragments and/or analogs or homologs in combination with other therapeutic agents to treat viral infections, particularly HIV infection. Further, the invention encompasses novel pharmaceutical compositions comprising DP-178 or DP-107 and at least one other therapeutic agent.

2. BACKGROUND OF THE INVENTION

2.1. The Human Immunodeficiency Virus

The human immunodeficiency virus (HIV) is a pathogenic retrovirus and the causative agent of acquired immune deficiency syndrome (AIDS) and related disorders (Barre-

virus (Dalgleish, A. et al., 1984, Nature 312: 767-768, Maddon et al., 1986, Cell 47:333-348). Viral entry into cells is dependent upon gp120 binding the cellular CD-4+ receptor molecules, while gp41 anchors the envelope glycoprotein complex in the viral membrane (McDougal, J.S. et al., 1986, Science 231:382-385; Maddon, P.J. et al., 1986, Cell 47:333-348) and thus explains HIV's tropism for CD-4+ cells.

2.2. HIV Treatment

HIV infection is pandemic and HIV associated diseases represent a major world health problem. Although considerable effort is being put into the successful design of effective therapeutics, currently no curative anti-retroviral drugs against AIDS exist. In attempts to develop such drugs, several stages of the viral life cycle have been considered targets for therapeutic intervention (Mitsuya, H. et al., 1991, FASEB J. 5:2369-2381). Intervention could potentially inhibit the binding of HIV to cell membranes, the reverse transcription of HIV RNA genome into DNA or the exit of the virus from the host cell and infection of new cellular targets.

Attempts are being made to develop drugs which can inhibit viral entry into the cell, the earliest stage of HIV infection. Here, the focus has been on CD-4+, the cell surface receptor for HIV. For example, recombinant soluble CD-4 has been shown to block HIV infectivity by binding to viral particles before they encounter CD-4 molecules embedded in cell membranes (Smith, D.H. et al., 1987, Science 238:1704-1707). Certain primary HIV-1 isolates are relatively less sensitive to inhibition by recombinant CD-4 (Daar, E. et al., 1990, Ann. Int. Med. 112:247-253). In addition, recombinant soluble CD-4 clinical trials have produced inconclusive results (Schooley, R. et al., 1990, Ann. Int. Med. 112:247-253; Kahn, J.O. et al., 1990, Ann. Int. Med. 112:254-261; Yarchoan, R. et al., 1989, Proc. Vth Int. Conf. on AIDS, p564, MCP 137).

nucleoside analog and a uridine phosphorylase inhibitor has been developed for the treatment of HIV, see Sommadossi, J.P. et al., U.S. Patent No. 5,077,280. Although these specific therapies may prove to be beneficial, combination therapy in general has the potential for antagonism as demonstrated in vitro with azidothymidine (AZT) and ribavirin. See U.S. Patent No. 4,950,652. Moreover, combination therapy is potentially problematic given the high toxicity of most anti-HIV therapeutics and their low level of effectiveness. Thus, there is a need for a combination therapy which is effective yet non-toxic.

The present invention provides a novel combination therapy based on the use of viral fusion inhibitors (DP-178 and DP-107, etc.) in combination with other antivirals. DP-178 and DP-107 are both novel therapeutics in that they prevent the virus from fusing with the cell, thereby very effectively preventing cell to cell transmission of the virus. In addition, DP-178 and DP-107 have proven to be non-toxic in in vitro studies and in animals. The present invention provides the first reported use of such peptides in combination with another antiviral or any other therapeutic agent.

3. SUMMARY OF THE INVENTION

The present invention relates to methods of treating or preventing viral infections, in particular HIV infections, in mammals, including humans, by administering an effective amount of DP-178, or a pharmaceutically acceptable derivative thereof in combination with at least one other therapeutic agent.

The present invention also relates to methods of treating or preventing viral infections, in particular HIV infections, in mammals, including humans, by administering an effective amount of DP-107 or pharmaceutically acceptable derivatives thereof in combination with at least one other therapeutic agent.

the novel antiviral combinations provide a means for circumventing the development of viral resistance to a single therapy, thereby providing the clinician with a more efficacious treatment.

Another aspect of the invention encompasses pharmaceutical compositions and formulations for treating or preventing viral infections, in particular HIV infections, wherein said compositions comprise an effective amount of DP-178, DP-107, or a pharmaceutically acceptable derivative thereof, at least one additional therapeutic agent and a pharmaceutically acceptable carrier.

Therapeutic agents to be used in combination with DP-178, DP-107 or a pharmaceutically acceptable derivative thereof encompass a wide variety of known treatments. Preferably, the combinations employ DP-107 or DP-178 in combination with agents with a different mode of attack. Such agents include but are not limited to: antivirals, such as cytokines, e.g., rIFN α, rIFN β, rIFN γ; inhibitors of reverse transcriptase, e.g., AZT, 3TC, D4T, ddI, and other dideoxynucleosides or dideoxyfluoronucleosides; inhibitors of viral mRNA capping, such as ribavirin; inhibitors of HIV protease, such as ABT-538 and MK-639; amphotericin B as a lipid-binding molecule with anti-HIV activity; and castanospermine as an inhibitor of glycoprotein processing.

Thus, the present invention provides an improved antiviral therapy for treating a broad spectrum of viruses including HIV.

The present invention also provides combinational therapy which yields improved efficacy over either agent used as a single-agent therapy.

In addition, the invention provides combinational therapy which allows for reduced toxicity of DP-178 and DP-107 and/or the therapeutic agent with which the peptides are used; thereby providing a higher therapeutic index.

The instant invention provides a combinational therapy which provides a means for circumventing the development of viral resistance to a single therapy.

indicate antagonism and values equal to 1 indicate additive effects.

The results of these assays are also analyzed using the method of Pritchard and Shipman (Pritchard and Shipman, 1990, *Antiviral Research* 14: 181-206). This computer program through three dimensional graphic analysis of the results allows for a determination of a synergistic or antagonistic interaction between the antiviral agents.

The term "pharmaceutically acceptable carrier" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredient, is chemically inert and is not toxic to the patient to whom it is administered.

As used herein the term "pharmaceutically acceptable derivative" refers to any homolog, analog, or fragment corresponding to the DP-178 or DP-107 peptides as described in Section 5.1.2. infra which exhibits antiviral activity and is relatively non-toxic to the subject.

The term "therapeutic agent" refers to any molecule, compound or treatment, preferably an antiviral, that assists in the treatment of a viral infection or the diseases caused thereby.

Peptides are defined herein as organic compounds comprising two or more amino acids covalently joined by peptide bonds. Peptides may be referred to with respect to the number of constituent amino acids, i.e., a dipeptide contains two amino acid residues, a tripeptide contains three, etc. Peptides containing ten or fewer amino acids may be referred to as oligopeptides, while those with more than ten amino acid residues are polypeptides.

Peptide sequences defined herein are represented by one-letter symbols for amino acid residues as follows:

A (alanine)
R (arginine)
N (asparagine)
D (aspartic acid)
C (cysteine)

50% compared to the untreated control. Control: the level of RT produced by untreated cell cultures infected with the same level of virus as treated cultures.

FIG. 3. Inhibition of HIV-1 and HIV-2 cell-free virus infection by the synthetic peptide DP-178 (SEQ ID:1). IC50: concentration of peptide necessary to inhibit RT production by 50% compared to the untreated control. Control: Level of RT produced by untreated cell cultures infected with the same level of virus as treated cultures.

FIG. 4. Cytotoxicity study of DP-178 (SEQ ID:1) and DP-116 (SEQ ID:9) on CEM cells. Cell proliferation data is shown.

FIG. 5A-C. DP178-derived peptide antiviral data. The peptides listed herein were derived from the region surrounding the HIV-1 BRU isolate DP178 region (e.g., gp41 amino acid residues 615-717). In instances where peptides contained DP178 point mutations, the mutated amino acid residues are shown with a shaded background. In instances in which the test peptide has had an amino and/or carboxy-terminal group added or removed (apart from the standard amido- and acetyl-blocking groups found on such peptides), such modifications are indicated.

FIG. 5A. The column to the immediate right of the name of the test peptide indicates the size of the test peptide and points out whether the peptide is derived from a one amino acid peptide "walk" across the DP178 region. The next column to the right indicates whether the test peptide contains a point mutation, while the column to its right indicates whether certain amino acid residues have been added to or removed from the DP178-derived amino acid sequence.

FIG 5B. The column to the immediate right of the test peptide name indicates whether the peptide represents a DP178 truncation, the next column to the right points out whether the peptide contains a point mutation, and the column to its right indicates whether the peptide contains amino acids which have been added to or removed from the DP178 sequence itself.

therapeutic agent are administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially, including cycling therapy. Cycling therapy involves the administration of a first antiviral compound for a period of time, followed by the administration of a second antiviral compound for a period of time and repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies. The invention also encompasses cycling therapy which comprises the administration of a first peptide of the present invention, followed by another antiviral, followed by another peptide of the present invention, etc., such that both viral fusion inhibitors DP-107 and DP-178 or derivatives thereof are used in combination with other antivirals. The invention also encompasses the use of a combination of the peptides, e.g., DP-107 in combination with DP-178.

Administration of DP-178, DP-107 or a pharmaceutically acceptable derivative thereof and one or more therapeutics "in combination" includes presentations in which both agents are administered together as a therapeutic mixture, and also procedures in which the two agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the drugs given first, followed by the second.

The Applicants' novel therapy involves the use of peptides which inhibit viral fusion and cell to cell transmission of the virus in combination with another therapeutic. Without being limited by theory, the present invention is based, in part, on the belief that HIV is believed to be replicating 24 hours a day from the first day of infection. Therefore it may be beneficial to use antiviral treatment at different stages of the viral infection.

The combinations disclosed herein present the first known use of viral fusion inhibitors, acting at the first

peptides include DP-178, a gp41 derived 36 amino acid peptide, fragments and/or analogs of DP-178, and peptides homologous to DP-178. In addition, these peptides may include peptides exhibiting antiviral activity which are analogous to DP-107, a 38 amino acid peptide, corresponding to residues 558 to 595 of the HIV-1_{LAI} transmembrane gp41 protein, and which are present in other enveloped viral proteins. The use of the peptides of the invention as inhibitors of non-human and human and retroviral, especially HIV transmission are detailed herein and in U.S. Patent Application Serial No. 08/073,028, filed June 7, 1993, U.S. Patent Application Serial No. 08/264,531, filed June 23, 1994, U.S. Patent Application Serial No. 08/255,208, filed June 7, 1994, U.S. Patent Application Serial No. 08/360,107, filed December 20, 1994, U.S. Patent Application Serial No. 08/374,666, filed January 27, 1995, U.S. Patent Application Serial No. 08/470,896, filed June 6, 1995, and U.S. Patent Application Serial No. 08/485,264, filed June 7, 1995, which are incorporated by reference herein in their entirety.

While not limited to any theory of operation, the following model is proposed to explain the potent anti-HIV activity of DP-178. In the viral protein, gp41, DP-178 corresponds to a putative α-helix region located in the C-terminal end of the gp41 ectodomain, and appears to associate with a distal site on gp41 whose interactive structure is influenced by the leucine zipper motif, a coiled-coil structure, referred to as DP-107. The association of these two domains may reflect a molecular linkage or "molecular clasp" intimately involved in the fusion process. It may be that the leucine zipper motif is involved in membrane fusion while the C-terminal α-helix motif serves as a molecular safety mechanism to regulate the availability of the leucine zipper during virus induced membrane fusion.

When synthesized as peptides both DP-107 and DP-178 are potent inhibitors of HIV infection and fusion, probably by virtue of their ability to form complexes with viral gp41 and interfere with its fusogenic process; e.g., during the

represent a carboxyl (-COOH) group. Alternatively, as described below, "X" and/or "Z" may represent a hydrophobic group, an acetyl group, a FMOC group, an amido group, or a covalently attached macromolecule.

DP-107 is a 38 amino acid peptide corresponding to residues 558 to 595 of HIV-1_{LAI} transmembrane (TM) gp41 protein, which exhibits potent antiviral activity. DP-107 is an HIV-1-derived antiviral peptide and may also be found in other, non-HIV-1 envelope viruses. DP-107 is more fully described in Applicant's co-pending U.S. Patent Applications Ser. No. 08/470,896, filed June 6, 1995, Ser. No. 08/374,666, filed January 27, 1995, Ser. No. 08/264,531, filed June 23, 1994, and Ser. No. 08/255,208, filed June 7, 1994, which are incorporated herein by reference in their entirety.

Deletions of DP107 or DP178 truncations are also within the scope of the invention. Such deletions consist of the removal of one or more amino acids from the DP107 or DP107-like peptide sequence, with the lower limit length of the resulting peptide sequence being 4 to 6 amino acids. Such deletions may involve a single contiguous or greater than one discrete portion of the peptide sequences. One or more such deletions may be introduced into DP107 or DP107 truncations, as long as such deletions result in peptides which may still be recognized by the 107x178x4, ALLMOTI5 or PLZIP search motifs described herein, or may, alternatively, exhibit antifusogenic or antiviral activity, or exhibit the ability to modulate intracellular processes involving coiled-coil peptide structures.

DP107 and DP107 truncations are more fully described in Applicants' co-pending U.S. Patent Application Serial No. 08/374,666, filed January 27, 1995, and which is incorporated herein by reference in its entirety.

TABLE II
DP-178 (SEQ ID:1) AMINO TRUNCATIONS

X-NWF-Z
X-WNWF-Z
X-LWNWF-Z
X-SLWNWF-Z
X-ASLWNWF-Z
X-WASLWNWF-Z
X-KWASLWNWF-Z
X-DKWASLWNWF-Z
X-LDKWASLWNWF-Z
X-ELDKWASLWNWF-Z
X-LELDKWASLWNWF-Z
X-LLELDKWASLWNWF-Z
X-ELLELDKWASLWNWF-Z
X-QELLELDKWASLWNWF-Z
X-EQELLELDKWASLWNWF-Z
X-NEQELLELDKWASLWNWF-Z
X-KNEQELLELDKWASLWNWF-Z
X-EKNEQELLELDKWASLWNWF-Z
X-QEKNEQELLELDKWASLWNWF-Z
X-QQEKNEQELLELDKWASLWNWF-Z
X-NQQEKNEQELLELDKWASLWNWF-Z
X-QNQQEKNEQELLELDKWASLWNWF-Z
X-SQNQQEKNEQELLELDKWASLWNWF-Z
X-ESQNQQEKNEQELLELDKWASLWNWF-Z
X-EESQNQQEKNEQELLELDKWASLWNWF-Z
X-IEESQNQQEKNEQELLELDKWASLWNWF-Z
X-LIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-SLIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-HSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-IHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-LIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-SLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-TSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z
X-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z

The one letter amino acid code is used.

Additionally,

"X" may represent an amino group, a hydrophobic group, including but not limited to carbobenzoxyl, dansyl, or T-butyloxycarbonyl; an acetyl group; a 9-fluorenylmethoxy-carbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

"Z" may represent a carboxyl group; an amido group; a T-butyloxycarbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

acids in length. One or more insertions may be introduced into DP-178 (SEQ ID:1), DP-178 fragments, analogs and/or DP-178 homologs.

Deletions of DP-178 (SEQ ID:1), DP-178 fragments, analogs, and/or DP-178 homologs are also within the scope of the invention. Such deletions consist of the removal of one or more amino acids from the DP-178 or DP-178-like peptide sequence, with the lower limit length of the resulting peptide sequence being 4 to 6 amino acids. Such deletions may involve a single contiguous or greater than one discrete portion of the peptide sequences.

The peptides of the invention may further include homologs of DP-178 (SEQ ID:1) and/or DP-178 truncations which exhibit antiviral activity. Such DP-178 homologs are peptides whose amino acid sequences are comprised of the amino acid sequences of peptide regions of other (i.e., other than HIV-1_{LAI}) viruses that correspond to the gp41 peptide region from which DP-178 (SEQ ID:1) was derived. Such viruses may include, but are not limited to, other HIV-1 isolates and HIV-2 isolates. DP-178 homologs derived from the corresponding gp41 peptide region of other (i.e., non HIV-1_{LAI}) HIV-1 isolates may include, for example, peptide sequences as shown below.

NH_2-YTNTIYTLLEESQNQQEKNEQELLELDKWASLWNWF-COOH (DP-185; SEQ ID:3);

NH_2-YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF-COOH (SEQ ID:4);

NH_2-YTSLIYSLLEKSQIQQEKNEQELLELDKWASLWNWF-COOH (SEQ ID:5).

SEQ ID:3 (DP-185), SEQ ID:4, and SEQ ID:5 are derived from HIV-1_{SF2}, HIV-1_{RP}, and HIV-1_{MN} isolates, respectively. Underlined amino acid residues refer to those residues that differ from the corresponding position in the DP-178 (SEQ ID:1) peptide. One such DP-178 homolog, DP-185 (SEQ ID:3),

TABLE III

HIV-2_{NIHZ} DP-178 homolog carboxy truncations.

X-LEA-Z
X-LEAN-Z
X-LEANI-Z
X-LEANIS-Z
X-LEANISQ-Z
X-LEANISQS-Z
X-LEANISQSL-Z
X-LEANISQSLE-Z
X-LEANISQSLEQ-Z
X-LEANISQSLEQA-Z
X-LEANISQSLEQAQ-Z
X-LEANISQSLEQAQI-Z
X-LEANISQSLEQAQIQ-Z
X-LEANISQSLEQAQIQQ-Z
X-LEANISQSLEQAQIQQE-Z
X-LEANISQSLEQAQIQQEK-Z
X-LEANISQSLEQAQIQQEKN-Z
X-LEANISQSLEQAQIQQEKNM-Z
X-LEANISQSLEQAQIQQEKNMY-Z
X-LEANISQSLEQAQIQQEKNMYE-Z
X-LEANISQSLEQAQIQQEKNMYEL-Z
X-LEANISQSLEQAQIQQEKNMYELQ-Z
X-LEANISQSLEQAQIQQEKNMYELQK-Z
X-LEANISQSLEQAQIQQEKNMYELQKL-Z
X-LEANISQSLEQAQIQQEKNMYELQKLN-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNS-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSW-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWD-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDV-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVF-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFT-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTN-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNW-Z
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL-Z

The one letter amino acid code is used.

Additionally,

"X" may represent an amino group, a hydrophobic group, including but not limited to carbobenzoxyl, dansyl, or T-butyloxycarbonyl; an acetyl group; a 9-fluorenylmethoxy-carbonyl (FMOC) group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

"Z" may represent a carboxyl group; an amido group; a T-butyloxycarbonyl group; a macromolecular carrier group including but not limited to lipid-fatty acid conjugates, polyethylene glycol, or carbohydrates.

5.1.3. Preparation Of DP-178 And DP-107

The peptides of the invention may be synthesized or prepared by techniques well known in the art. See, for example, Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman and Co., NY, which is incorporated herein by reference in its entirety. Short peptides, for example, can be synthesized on a solid support or in solution. Longer peptides amy be made using recombinant DNA techniques. Here, the nucleotide sequences encoding the peptides of the invention may be synthesized, and/or cloned, and expressed according to techniques well known to those of ordinary skill in the art. See, for example, Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, Vols. 1-3, Cold Spring Harbor Press, NY.

The peptides of the invention may alternatively be synthesized such that one or more of the bonds which link the amino acid residues of the peptides are non-peptide bonds. These alternative non-peptide bonds may be formed by utilizing reactions well known to those in the art, and may include, but are not limited to imino, ester, hydrazide, semicarbazide, and azo bonds, to name but a few. In yet another embodiment of the invention, peptides comprising the sequences described above may be synthesized with additional chemical groups present at their amino and/or carboxy termini, such that, for example, the stability, bioavailability, and/or inhibitory activity of the peptides is enhanced. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups, may be added to the peptides' amino termini. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the peptides' amino termini. (See "X" in Tables I to IV, above.) Additionally, the hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to the peptides' carboxy termini. (See "Z" in Tables I to IV, above.) Further, the peptides of the invention may be synthesized such that their steric configuration is altered. For example, the D-isomer of one or more of the amino acid

Non retroviral viruses whose transmission may be inhibited by the peptides of the invention include, but are not limited to human respiratory syncytial virus, canine distemper virus, newcastle disease virus, human parainfluenza virus, and influenza viruses.

The invention further encompasses the treatment of the above retroviral and non-retroviral viruses using the peptides in combination therapy.

5.2. Antivirals To Be Used In Combination With DP-178 Or DP-107

According to the present invention, DP-178 or DP-107, a virus fusion inhibitor, may be used in combination with other therapeutic agents to enhance its antiviral effect achieved. Preferably DP-178 or DP-107 is used in combination with another antiviral agent. Such additional antiviral agents which may be used with DP-178 or DP-107 include but are not limited to those which function on a different target molecule involved in viral replication, e.g., reverse transcriptase inhibitors, viral protease inhibitors, glycosylation inhibitors; those which act on a different target molecule involved in viral transmission; those which act on a different loci of the same molecule; and those which prevent or reduce the occurrence of viral resistance. One skilled in the art would know of a wide variety of antiviral therapies which exhibit the above modes of activity.

DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof can also be used in combination with retrovirus inhibitors, such as nucleoside derivatives. Nucleoside derivatives are modified forms of purine and pyrimidine nucleosides which are the building blocks of RNA and DNA. Many of the nucleoside derivatives under study as potential anti-HIV medications result in premature termination of viral DNA replication before the entire genome has been transcribed. These derivatives lack 3' substituents that can bind to subsequent nucleosides and result in chain termination. Nucleoside derivatives such as 3'azido-3'-

without loss of antiviral activity because of the use of the antiviral peptides. Moreover, such a combination reduces or avoids viral resistance.

Preferred combinations of antiviral peptides and nucleoside derivatives within the scope of the present invention include an effective amount of DP-107, DP-178 or a pharmaceutically acceptable derivative thereof and an effective amount of AZT to treat HIV infection; and an effective amount of DP-107, DP-178 or a pharmaceutically acceptable derivative thereof and an effective amount of ddI.

According to the present invention, DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof can also be used in combination with uridine phosphorylase inhibitors, including but not limited to acyclouridine compounds, including benzylacyclouridine (BAU); benzyloxybenzylacyclouridine (BBAU); aminomethyl-benzylacyclouridine (AMBAU); aminomethyl-benzyloxybenzylacyclouridine (AMB-BAU); hydroxymethyl-benzylacyclouridine (HMBAU); and hydroxymethyl-benzyloxybenzylacyclouridine (HMBBAU).

According to the present invention, DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof can also be used in combination with cytokines or cytokine inhibitors, including but not limited to rIFN α, rIFN β, rIFN γ, inhibitors of TNFα, and MNX-160. Human rIFN-αA (>108 IU/mg) and rIFN γ (1.4 x 108 IU/mg) can be obtained from Hoffman LaRoche. Human rIFN β Ser 17 (1.0 x 108 IU/mg) are obtained from Triton Biosciences. Reference standards are obtained from the World Health Organization (human IFNα WHO standard B,69,19 and human IFN β, WHO no. G-023-902-527, or the National Institute of Allergy and Infectious Disease (human γ, National Institute of Health no. G-023-901-530.

According to the present invention, DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof can be used in combination with viral protease inhibitors, including but not limited to, MK-639 (Merck), Invirase (saquinavir, Roche), ABT-538 (Abbott, CAS Reg. No. 155213-67-5), AG1343, VX0478

agents that are active against a variety of lipid -enveloped viruses, including HIV. Although amphotericin exhibits severe in vivo toxicities, the methyl ester form of this drug also exhibits anti-HIV activity and has a low cellular toxicity profile in vitro. Therefore amphotericin B or its methyl ester can be used in combinational therapy with DP-178, DP-107 or a pharmaceutical derivative thereof. This combination allows the clinician to employ a lower i.e., less toxic dose of ether Amphotericin B or its methyl ester without concern for loss of antiviral activity since it is used in conjunction with the antiviral peptides DP-178 or DP-107.

According to the present invention, DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof can also be used in combination with inhibitors of glycoprotein processing, such as castonospermine (Boehringer Mannheim). Castanospermine is a plant alkaloid which inhibits glycoprotein processing, and acts as an anti-HIV since HIV contains two heavily glycosylated proteins, gp120 and gp41. Protein glycosylation plays an important role in gp120 interaction with CD4. Under conditions of infection by progeny virions synthesized in the presence of castanospermine the infectivity of HIV was attenuated. Therefore it is likely that DP-178, DP-107 or a pharmaceutically acceptable derivative thereof in combination with castanospermine would act synergistically to inhibit viral entry and hence attenuate infection.

Preferred combinations to be used within the methods of treating HIV include the use of an effective amount of DP-107, DP-178 or a pharmaceutically acceptable derivative thereof and an effective amount of ddI; the use of an effective amount of DP-107, DP-178 or a pharmaceutically acceptable derivative thereof and an effective amount of 3TC; and the use of an effective amount of DP-107, DP-178 or a pharmaceutically acceptable derivative thereof and an effective amount ribavirin.

by any appropriate means including but not limited to injection (e.g., intravenous, intraperitoneal, intramuscular, subcutaneous, etc.), by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and vaginal epithelial linings, nasopharyngeal mucosa, intestinal mucosa, etc.); orally, transdermally or any other means available within the pharmaceutical arts.

5.3. Pharmaceutical Formulations, Dosages And Modes Of Administration

5.3.1. Pharmaceutical Compositions

The pharmaceutical compositions of the invention which are useful in the treatment or prevention of viral infections in humans contain as an active agent DP-178, DP-107 or a pharmaceutically acceptable derivative thereof, and at least one other therapeutic agent, such as another antiviral. The pharmaceutical compositions of the present invention provide combinational therapy that may have either additive and/or synergistic effects.

Preferably, the pharmaceutical compositions containing DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof also contain at least one other antiviral agent, such as reverse transcriptase inhibitors, protease inhibitor, inhibitors of mRNA processing, inhibitors of protein glycosylation and inhibitors of viral fusion. Such agents include but are not limited to nucleoside analogs or chain terminators (e.g., dideoxynucleosides).

Additional suitable therapeutic agents which may be used in combinational therapy with DP-178 or DP-107 or a pharmaceutically acceptable derivative thereof within the scope of the invention include but are not limited to 2-deoxy-D-glucose (2-dGlc), deoxynojirimycin, acycloguanosine, ribavirin (virazole), rifampicin (rifadin), adamantidine, rifabutine, ganciclover, (DHPG), fluoroiodoaracytosine, idoxurine, trifluorothymidine, adenine arabinoside (ara-A), ara-AMP, bromovinyldeoxyuridine, bromovinylarauracil (BV-araU by Bristol-Meyers Squibb (1-beta-D-arabinofuranoside-E-5-[2-

prevention of virus transmission from mother to infant and other settings where the likelihood of HIV transmission exists, such as, for example, accidents in health care settings wherein workers are exposed to HIV-containing blood products. The peptides of the invention in such cases may serve the role of a prophylactic vaccine, wherein the host raises antibodies against the peptides of the invention, which then serve to neutralize HIV viruses by, for example, inhibiting further HIV infection. Administration of the peptides of the invention as a prophylactic vaccine, therefore, would comprise administering to a host a concentration of peptides effective in raising an immune response which is sufficient to neutralize HIV, by, for example, inhibiting HIV ability to infect cells. The exact concentration will depend upon the specific peptide to be administered, but may be determined by using standard techniques for assaying the development of an immune response which are well known to those of ordinary skill in the art. The peptides to be used as vaccines are usually administered intramuscularly.

The peptides may be formulated with a suitable adjuvant in order to enhance the immunological response. Such adjuvants may include, but are not limited to mineral gels such as aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols, polyanions; other peptides; oil emulsions; and potentially useful human adjuvants such as BCG and Corynebacterium parvum. Many methods may be used to introduce the vaccine formulations described here. These methods include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, and intranasal routes.

Effective dosages of the peptides of the invention to be administered may be determined through procedures well known to those in the art which address such parameters as biological half-life, bioavailability, and toxicity. Given the data presented below in Section 6, DP-178, for example,

day 1 and daily thereafter (Ho, et al., 1995, Nature 373: 123-126). These recommended or known levels will preferably be lowered by 10% to 50% of the cited dosage after testing the effectiveness of these dosages in combination with DP-178, DP-107 or a pharmaceutically acceptable derivative, using the assays described in Section 5.4 infra. It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust therapy to lower dosage due to toxicity, bone marrow, liver or kidney dysfunctions or adverse drug-drug interaction. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response is not adequate (precluding toxicity).

A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-

considered to not contain HIV-1. Such an isolate may then be treated with one or more of the other DP-178 peptides of the invention, and subsequently be tested for its viral activity in order to determine the identify of the viral isolate.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections; transdermal, topical, vaginal and the like. Dosage forms include but are not limited to tablets, troches, dispersions, suspensions, suppositories, solutions, capsules, creams, patches, minipumps and the like.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in

glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration,the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily

compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

5.5. Assays For Antiviral Activity

The antiviral activity exhibited by the combination therapy of the invention may be measured, for example, by easily performed *in vitro* assays, such as those described below, which can test the peptides' ability to inhibit syncytia formation, or their ability to inhibit infection by cell-free virus. Using these assays, such parameters as the relative antiviral activity of the peptides, exhibit against a given strain of virus and/or the strain specific inhibitory activity of the peptide can be determined. A cell fusion assay may be utilized to test the peptides' ability to inhibit HIV-induced syncytia formation *in vitro*. Such an assay may comprise culturing uninfected CD-4$^+$ cells (such as Molt or CEM cells, for example) in the presence of

of such techniques. These references are incorporated by reference herein in its entirety.

5.5.1. Testing Of Antiviral Compounds Active At Different Stages Of HIV-1 Infection

Three separate in vitro assays for the study of antiviral compounds active at different stages of HIV infection (acute, co-cultivation, and chronic) are well known to those skilled in the art (Lambert et al., 1993, Antiviral Res. 21: 327-342). These assays can be used to assess the effects of DP-178, DP-107 or a pharmaceutically acceptable derivative thereof in combination with one of the described antiviral agents. All assays are carried out in triplicate in 24-well plates (Nunc.) 5-fold serial dilutions of inhibitor are made in 100% DMSO to yield 200 x final concentrations. Addition of 1/200 vol. of dilutions to culture wells resulted in a final concentration of 0.5% DMSO and the desired concentration of the inhibitor. Experiments are carried out either with dilutions of fixed ratio of the two inhibitors (i.e., 1:10 or 1:40, AZT:DP-178) or where the concentrations are varied.

First the acute infection assay models the rapid replication and cytopathic effects contributing to the loss of CD-4+ cells in vivo. Assay the treatment of acutely infected Molt4 cells to show the antiviral compounds are effective at inhibiting the spread of HIV-1 infection in T cells. For these assays, 3 x 10^4 uninfected Molt4 cells per well are infected with 50 TCIDs of HIV-1 (strain LA1). Stocks of inhibitors are prepared in 100% DMSO, and added on day 0, immediately after the 1.5 hour virus absorption period. Cultures are re-fed on days 1 and 4 with medium containing the same concentration of inhibitor. Samples are harvested on day 7.

Second, chronically infected cells, containing integrated provirus and exhibiting moderate to low levels of continuous virus expression, are likely to represent in vivo reservoirs of infectious virions, which ultimately contribute

where fa is the fraction affected by dose D, fu is the uninfected fraction, Dm is the dose required for 50% effect and m is the slope of the dose-effect curve. For mutually exclusive drugs (i.e. similar modes of action), both drugs alone and their parallel lines in the median effect plot. Mutually nonexclusive drugs (i.E. independent mode of action) will give parallel lines in the median effect plot, but in mixture will give a concave upward curve. If the agents are mutually exclusive α is 0, and if they are mutually nonexclusive, α is 1. Values obtained assuming mutual nonexclusiveness will always be slightly greater than mutually exclusive drugs. CI values of <1 indicate synergy, values >1 indicate antagonism and values equal to 1 indicate additive effects.

The combined drug effects are also calculated by the MacSynergy computer program (Pritchard and Shipman, 1990, *Antiviral Research* 14: 181-206). This computer program allows three-dimensional graphic analysis of drug-drug interactions. The amount of synergy observed with combinations of antiviral compounds is calculated by the MacSynergy program and is represented by a three-dimensional bar graph in which the percentage of drug interaction is plotted versus drug concentrations. The amount of synergy is represented by the heights of bars in the graph and antagonism is plotted as a negative value below the floor of the graph.

6. EXAMPLE: DP-178 (SEQ ID:1) IS A POTENT INHIBITOR OF HIV-1 INFECTION

In this example, DP-178 (SEQ ID:1) is shown to be a potent inhibitor of HIV-1 mediated CD-4$^+$ cell-cell fusion and infection by cell free virus. In the fusion assay, this peptide completely blocks virus induced syncytia formation at concentrations of from 1-10 ng/ml. In the infectivity assay the inhibitory concentration is somewhat higher, blocking infection at 90ng/ml. It is further shown that DP-178 (SEQ ID:1) shows that the antiviral activity of DP-178 (SEQ ID:1)

virus was added to 75μl AA5 cells at a concentration of 2 x 10^5/ml in a 96-well microtitre plate. Each virus dilution was tested in triplicate. Cells were cultured for eight days by addition of fresh medium every other day. On day 8 post infection, supernatant samples were tested for virus replication as evidenced by reverse transcriptase activity released to the supernatant. The $TCID_{50}$ was calculated according to the Reed and Muench formula (Reed, L.J. et al., 1938, Am. J. Hyg. 27:493-497). The titer of the HIV-1_{LAI} and HIV-1_{MN} stocks used for these studies, as measured on the AA5 cell line, was approximately 1.4 x 10^6 and 3.8 x 10^4 $TCID_{50}$/ml, respectively.

6.1.3. Cell Fusion Assay

Approximately 7 x 10^4 Molt cells were incubated with 1 x 10^4 CEM cells chronically infected with the HIV-1_{LAI} virus in 96-well plates (one-half area cluster plates; Costar, Cambridge, MA) in a final volume of 100μl culture medium as previously described (Matthews, T.J. et al., 1987, Proc. Natl. Acad. Sci. USA 84: 5424-5428). Peptide inhibitors were added in a volume of 10μl and the cell mixtures were incubated for 24 hr. at 37°C. At that time, multinucleated giant cells were estimated by microscopic examination at a 40x magnification which allowed visualization of the entire well in a single field.

6.1.4. Cell Free Virus Infection Assay

Synthetic peptides were incubated at 37°C with either 247 $TCID_{50}$ (for experiment depicted in FIG. 2), or 62 $TCID_{50}$ (for experiment depicted in FIG.3) units of HIV-1_{LAI} virus or 25 $TCID_{50}$ units of HIV-2_{NIH2} and CEM $CD4^+$ cells at peptide concentrations of 0, 0.04, 0.4, 4.0, and 40μg/ml for 7 days. The resulting reverse transcriptase (RT) activity in counts per minute was determined using the assay described, below, in Section 6.1.5. See, Reed, L.J. et al., 1938, Am. J. Hyg. 27: 493-497 for an explanation of $TCID_{50}$ calculations.

HIV-1_{SF2} virus were cocultivated overnight with uninfected Molt 4 cells. The results (FIG. 4) show that DP-178 (SEQ ID:1) afforded complete protection against each of the HIV-1 isolates down to the lowest concentration of DP-178 (SEQ ID:1) used. For HIV_{LAI} inhibition, the lowest concentration tested was 12.5ng/ml; for all other HIV-1 viruses, the lowest concentration of DP-178 (SEQ ID:1) used in this study was 100ng/ml. A second peptide, DP-180 (SEQ ID:2), containing the same amino acid residues as DP-178 (SEQ ID:1) but arranged in a random order exhibited no evidence of anti-fusogenic activity even at the high concentration of 40μg/ml (FIG. 4). These observations indicate that the inhibitory effect of DP-178 (SEQ ID:1) is primary sequence-specific and not related to non-specific peptide/protein interactions. The actual endpoint (i.e., the lowest effective inhibitory concentration) of DP-178 inhibitory action is within the range of 1-10 ng/ml.

The next series of experiments involved the preparation and testing of a DP-178 (SEQ ID:1) homolog for its ability to inhibit HIV-1-induced syncytia formation. As shown in FIG. 1, the sequence of DP-185 (SEQ ID:3) is slightly different from DP-178 (SEQ ID:1) in that its primary sequence is taken from the HIV-1_{SF2} isolate and contains several amino acid differences relative to DP-178 (SEQ ID:1) near the N terminus. As shown in FIG. 4, DP-185 (SEQ ID:3), exhibits inhibitory activity even at 312.5ng/ml, the lowest concentration tested.

The next series of experiments involved a comparison of DP-178 (SEQ ID:1) HIV-1 and HIV-2 inhibitory activity. As shown in FIG. 5, DP-178 (SEQ ID:1) blocked HIV-1-mediated syncytia formation at peptide concentrations below 1ng/ml. DP-178 (SEQ ID:1) failed, however, to block HIV-2 mediated syncytia formation at concentrations as high as 10μg/ml. This striking 4 log selectivity of DP-178 (SEQ ID:1) as an inhibitor of HIV-1-mediated cell fusion demonstrates an unexpected HIV-1 specificity in the action of DP-178 (SEQ ID:1). DP-178 (SEQ ID:1) inhibition of HIV-1-mediated cell

thus making DP-178 (SEQ ID:1) two logs more potent as a HIV-1 inhibitor than a HIV-2 inhibitor. This finding is consistent with the results of the fusion inhibition assays described, above, in Section 6.2.1, and further supports a significant level of selectivity (i.e., for HIV-1 over HIV-2).

7. EXAMPLE: THE HIV-1 INHIBITOR, DP-178 (SEQ ID:1) IS NON-CYTOTOXIC

In this Example, the 36 amino acid synthetic peptide inhibitor DP-178 (SEQ ID:1) is shown to be non-cytotoxic to cells in culture, even at the highest peptide concentrations (40μg/ml) tested.

7.1. Materials And Methods

Cell proliferation and toxicity assay: Approximately $3.8x10^5$ CEM cells for each peptide concentration were incubated for 3 days at 37°C in T25 flasks. Peptides tested were DP-178 (SEQ ID:1) and DP-116 (SEQ ID:9), as described in FIG. 1. The concentrations of each peptide used were 0, 2.5, 10, and 40μg/ml. Cell counts were taken at incubation times of 0, 24, 48, and 72 hours.

7.2. Results

Whether the potent HIV-1 inhibitor DP-178 (SEQ ID:1) exhibited any cytotoxic effects was assessed by assaying the peptide's effects on the proliferation and viability of cells in culture. CEM cells were incubated in the presence of varying concentrations of DP-178 (SEQ ID:1), and DP-116 (SEQ ID:9), a peptide previously shown to be ineffective as a HIV inhibitor (Wild, C. et al., 1992, Proc. Natl. Acad. Sci. USA 89:10,537-10,541). Additionally, cells were incubated in the absence of either peptide.

The results of the cytoxicity study demonstrate that DP-178 (SEQ ID:1) exhibits no cytotoxic effects on cells in culture. As can be seen, below, in Table V, even the proliferation and viability characteristics of cells cultured for 3 days in the presence of the highest concentration of

TABLE V

Peptide	Peptide Concentration µg/ml	% Viability at time (hours) 0	24	48	72
DP178 (SEQ ID:1)	40	98	97	95	97
	10	98	97	98	98
	2.5	98	93	96	96
DP116 (SEQ ID:9)	40	98	95	98	97
	10	98	95	93	98
	2.5	98	96	98	99
No Peptide	0	98	97	99	98

8. EXAMPLE: ANTI-VIRAL ACTIVITY OF DP-107 AND DP-178 PEPTIDE TRUNCATIONS AND MUTATIONS

The Example presented in this Section represents a study of the antiviral activity of DP107 and DP178 truncations and mutations. It is demonstrated that several of these DP107 and DP178 modified peptides exhibit substantial antiviral activity.

specific antiviral activity, in that none of the peptides tested on the HIV-2 NIHZ isolate demonstrated appreciable anti-HIV-2 antiviral activity.

Among the peptides listed in FIG. 5B, are test peptides representing the amino (T-4) and carboxy (T-3) terminal halves of DP178 were tested. The amino terminal peptide was not active (IC_{50}>400μg/ml) whereas the carboxy terminal peptide showed potent antiviral activity (IC_{50}= 3μg/ml). A number of additional test peptides also exhibited a high level of antiviral activity. These included, for example, T-61/T-102, T-217 to T-221, T-235, T-381, T-677, T-377, T-590, T-378, T-591, T-271 to T-272, T-611, T-222 to T-223 and T-60/T-224. Certain of the antiviral peptides contain point mutations and/or amino acid residue additions which vary from the DP178 amino acid sequence.

In FIG. 5C, point mutations and/or amino and/or carboxy-terminal modifications are introduced into the DP178 amino acid sequence itself. As shown in the figure, the majority of the test peptides listed exhibit potent antiviral activity.

Truncations of the DP107 peptide (referred to in FIG. 5 as T21) were also produced and tested, as shown in FIG. 6. FIG. 6 also presents data concerning blocked and unblocked peptides which contain additional amino acid residues from the gp41 region in which the DP107 sequence resides. Most of these peptides showed antiviral activity, as evidenced by their low IC_{50} values.

Thus, the results presented in this Section demonstrate that not only do the full length DP-107 and DP-178 peptides exhibit potent antiviral activity, but truncations of these peptides also possess substantial antiviral character.

9. EXAMPLE: POTENTIAL SIV DP178/DP107 ANALOGS: ANTIVIRL CHARACTERIZATION

In the Example presented herein, simian immunodeficiency virus (SIV) DP178-like peptides identified by utilizing the computer-assisted search motifs described above, were tested

What is claimed is:

1. A method of treating HIV infection in a subject which comprises administering an effective amount of DP-107 or a pharmaceutically acceptable derivative thereof and an effective amount of at least one therapeutic agent.

2. A method of treating HIV infection in a subject which comprises administering an effective amount of DP-178 or a pharmaceutically acceptable derivative thereof and an effective amount of at least one therapeutic agent.

3. The method of claims 1 or 2 wherein said therapeutic agent is an antiviral.

4. The method of claim 3 wherein said anti-HIV agent is a reverse transcriptase inhibitor, a viral protease inhibitor, a cytokine, a cytokine inhibitor, a glycosylation inhibitor or a viral mRNA processing inhibitor.

5. The method of claim 3 wherein said antiviral is a nucleoside analogue.

6. The method of claim 5 wherein said nucleoside analogue is AZT, ddI, ddC, ddA, d4T or 3TC.

7. The method of claim 3 wherein said antiviral is interferon-α, interferon-β or interferon-γ.

8. The method of claim 2 wherein said DP-178 derivative is a peptide of the group comprising T-624, T-636 to T-641, T-645 to T-650, T-652 to T-654, or T-656.

9. A method of inhibiting HIV replication comprising administering to the subject an effective amount of DP-107 or a pharmaceutically acceptable derivative thereof and an effective amount of at least one therapeutic agent.

19. The method of claim 1, 2, 9 or 10 wherein said administration is simultaneous.

20. The method of claim 1, 2, 9 or 10 wherein said administration is oral.

21. The method of claim 1, 2, 9 or 10 wherein said administration is parenteral.

22. The method of claim 21 wherein said administration is intravenous.

23. A pharmaceutically acceptable composition useful for the treatment of HIV infection which comprises an effective amount of DP-178 or a pharmaceutically acceptable derivative thereof, an effective amount another therapeutic agent and a pharmaceutically acceptable carrier.

24. A pharmaceutical composition useful for the treatment of HIV infection which comprises an effective amount of DP-107 or a pharmaceutically acceptable derivative thereof, an effective amount of another therapeutic agent and a pharmaceutically acceptable carrier.

25. The pharmaceutical composition of claim 23 or 24 wherein said therapeutic agent is an antiviral.

26. The pharmaceutical composition of claim 25 wherein said antiviral agent is a nucleoside analog.

27. The pharmaceutical composition of claim 26 wherein said nucleoside analog is AZT, ddI, ddC, ddA, or 3TC.

28. A method of treating HIV infection in a subject which comprises (a) administering an effective amount of DP-107, DP-178 or a pharmaceutically acceptable derivative thereof; (b) administering an effective amount of another

HIV1LAI (DP-178; SEQ ID:1)	YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF
HIV1SF2 (DP-185; SEQ ID:3)	YTNTIYNLLEESQNQQEKNEQELLELDKWASLWNWF
HIV1RF (SEQ ID:4)	YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF
HIV1MN (SEQ ID:5)	YTSLIYSLLEKSQTQQEKNEQELLELDKWASLWNWF
HIV2ROD (SEQ ID:6)	LEANISKSLEQAQIQQEKNMYELQKLNSWDIFGNWF
HIV2NIHZ (SEQ ID:7)	LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL
DP180 (SEQ ID:2)	SSESFTLLEQWNNWKLQLAEQWLEQINEKHYLEDIS
DP118 (SEQ ID:10)	QQLLDVVKRQQEMLRLTVWGTKNLQARVTAIEKYLKDQ
DP125 (SEQ ID:8)	CGGNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQ
DP116 (SEQ ID:9)	LQARILAVERYLKDQQQ

FIG.1

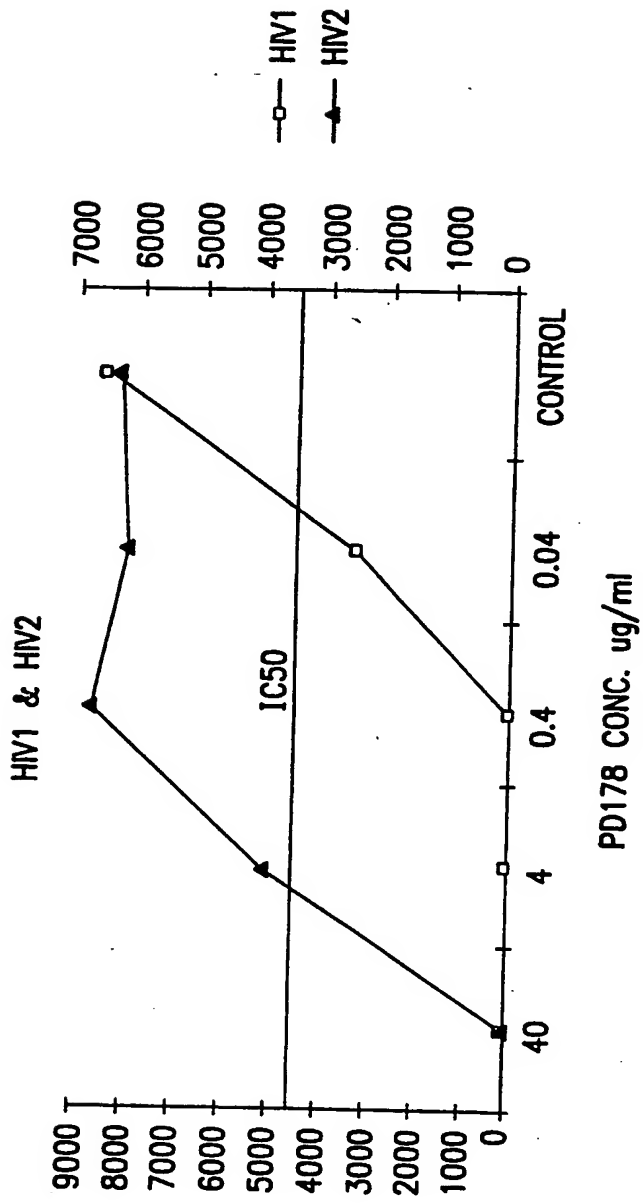

HIV1
HIV2
HIV1 & HIV2
IC50
PD178 CONC. ug/ml
CONTROL
0.04
0.4
4
40
0
1000
2000
3000
4000
5000
6000
7000
8000
9000

FIG.3

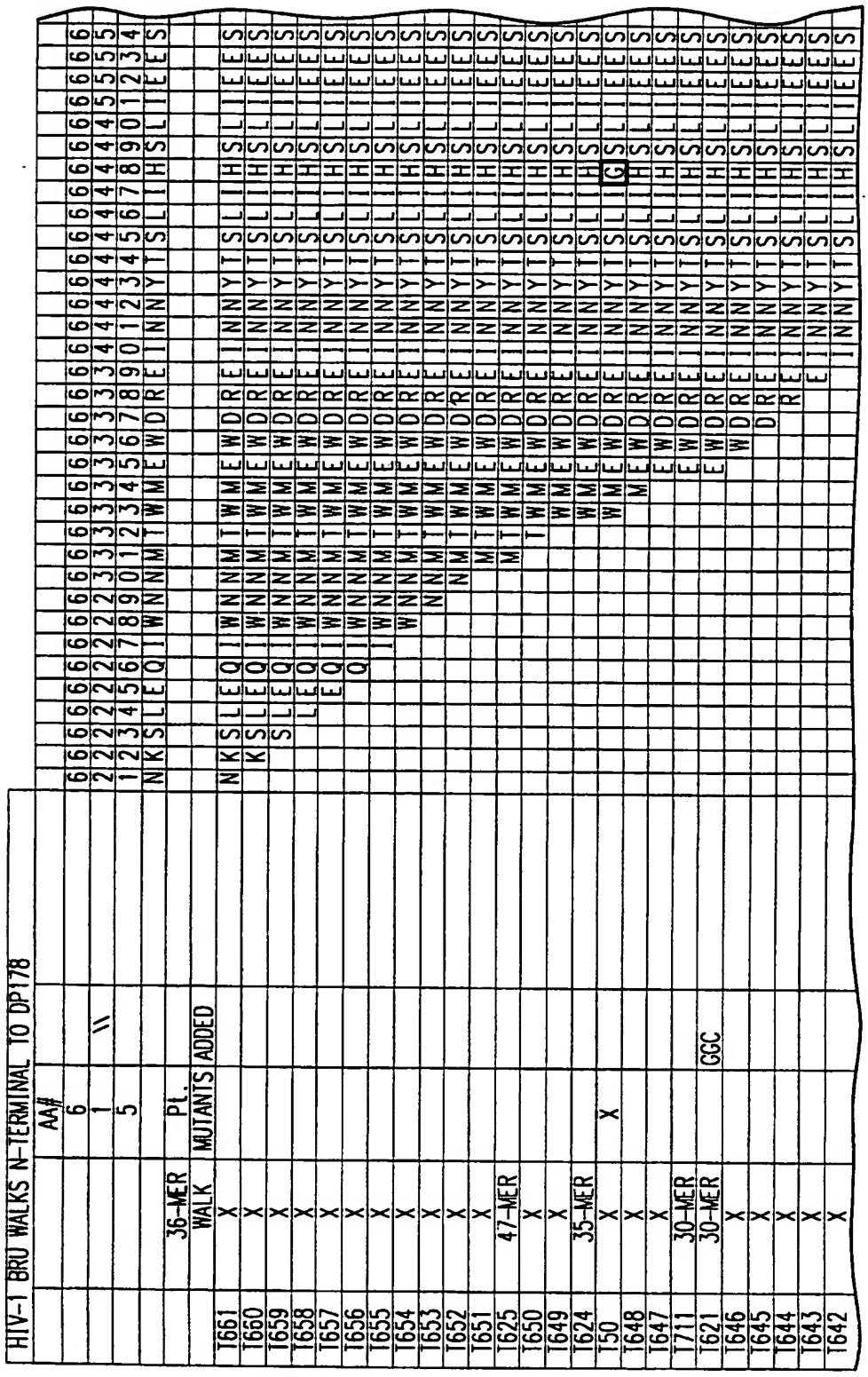

HIV-1 BRU WALKS N-TERMINAL TO DP178
AA#
WALK
MUTANTS ADDED
36-MER
Pt.
T661
T660
T659
T658
T657
T656
T655
T654
T653
T652
T651
T625
T650
T649
T624
T50
T648
T647
T711
T621
T646
T645
T644
T643
T642
47-MER
35-MER
30-MER
30-MER
GGC

FIG.5A(I)

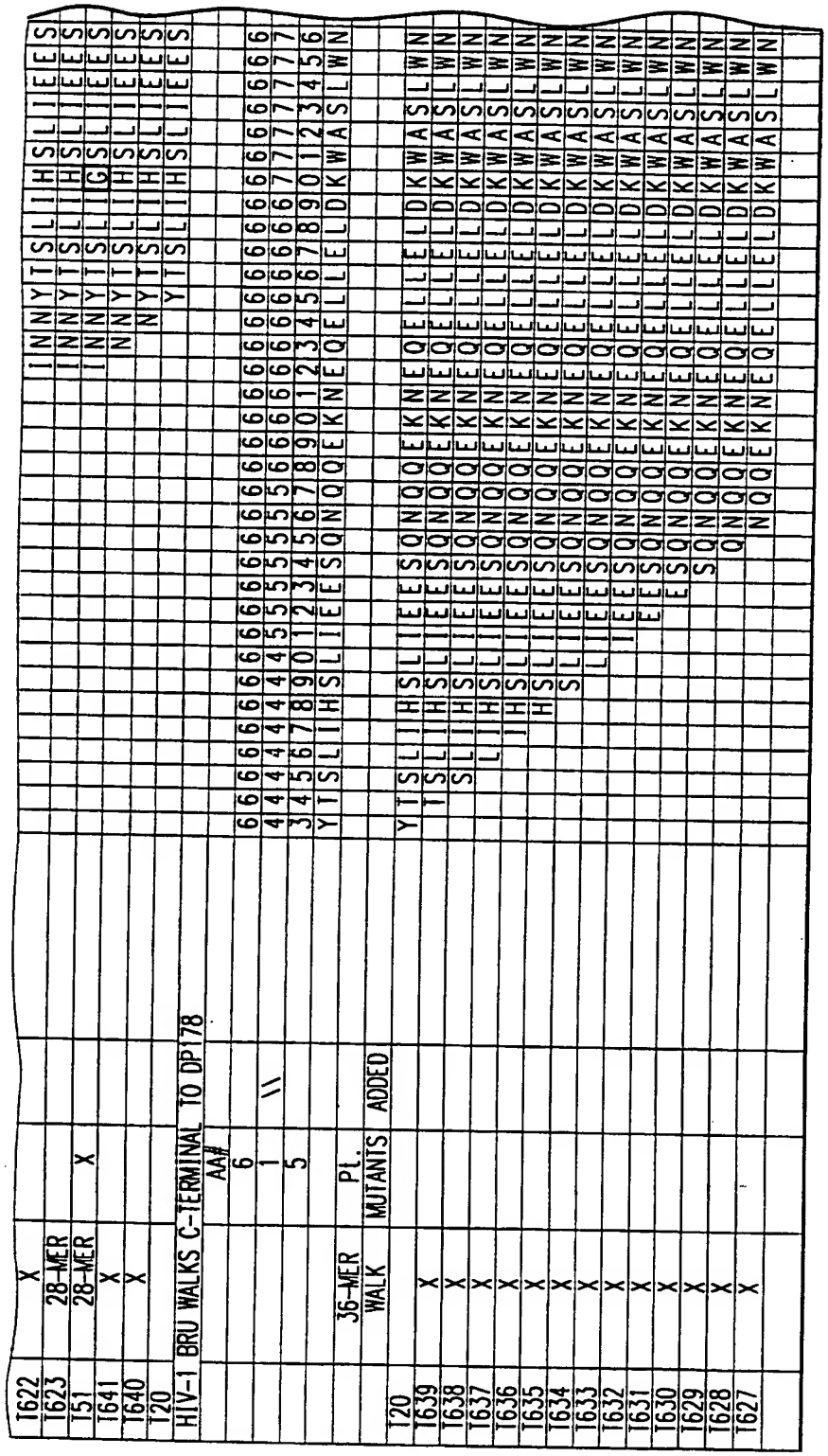

HIV-1 BRU WALKS C-TERMINAL TO DP178
AA#
36-MER WALK
Pt. MUTANTS
ADDED
28-MER
28-MER
T622
T623
I51
T641
T640
T20
T20
T639
T638
T637
T636
T635
T634
T633
T632
T631
T630
T629
T628
T627

FIG.5A(III)

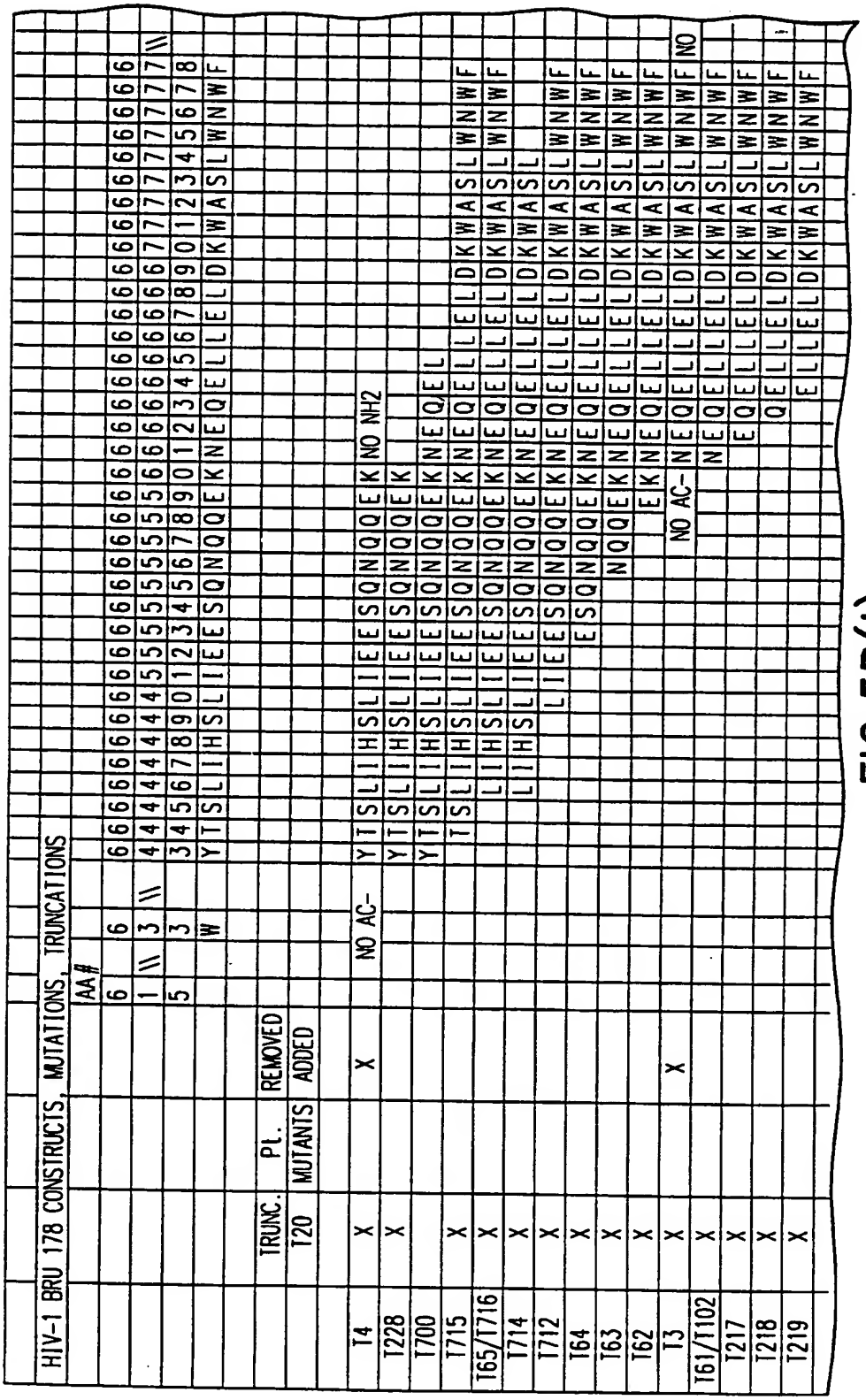

HIV-1 BRU 178 CONSTRUCTS, MUTATIONS, TRUNCATIONS
AA#
TRUNC.
Pt.
REMOVED
T20
MUTANTS
ADDED
T4
T228
T700
T715
T65/T716
T714
T712
T64
T63
T62
T3
T61/T102
T217
T218
T219
NO AC-
NO NH2
NO

FIG.5B(1)

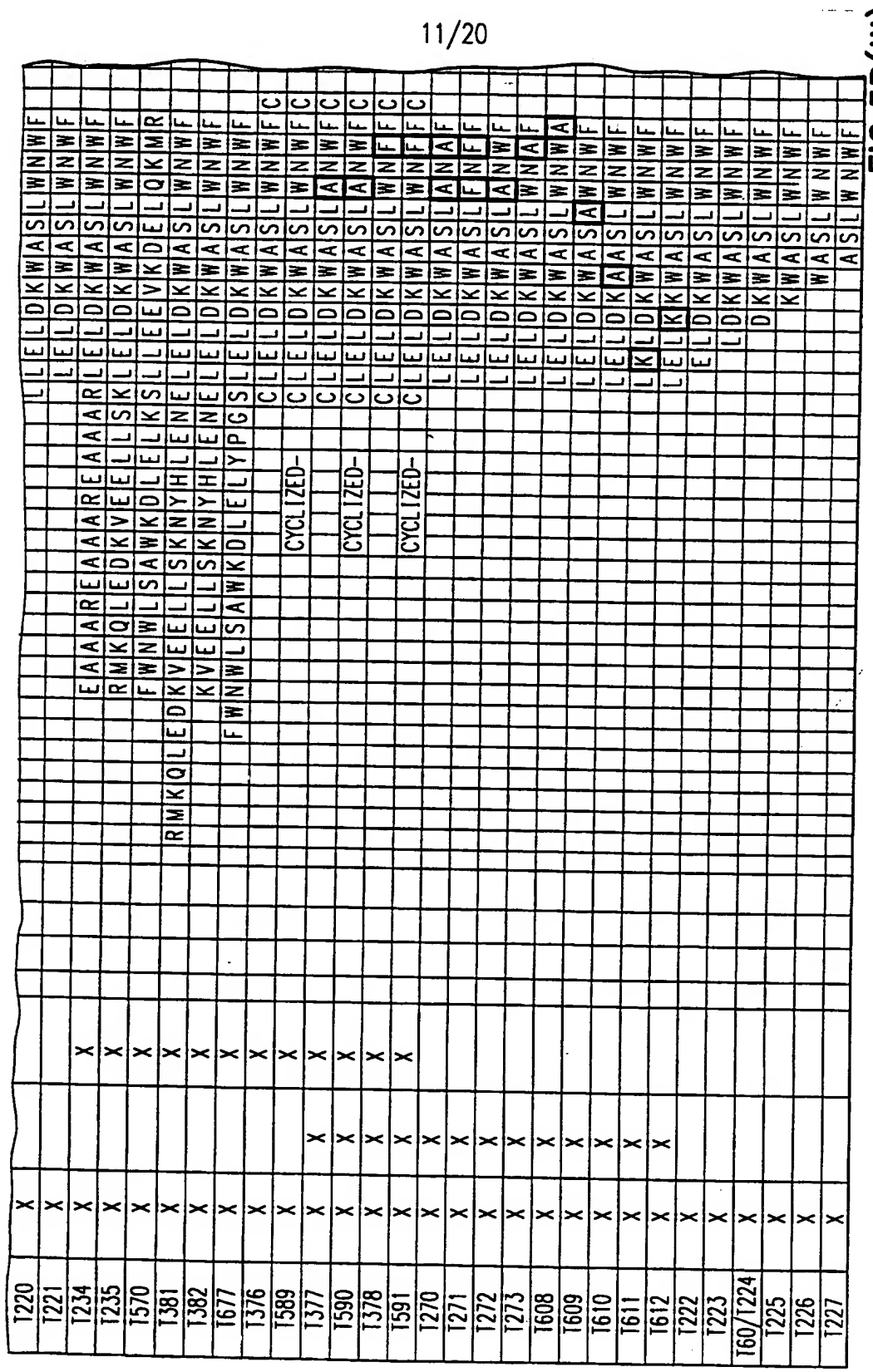
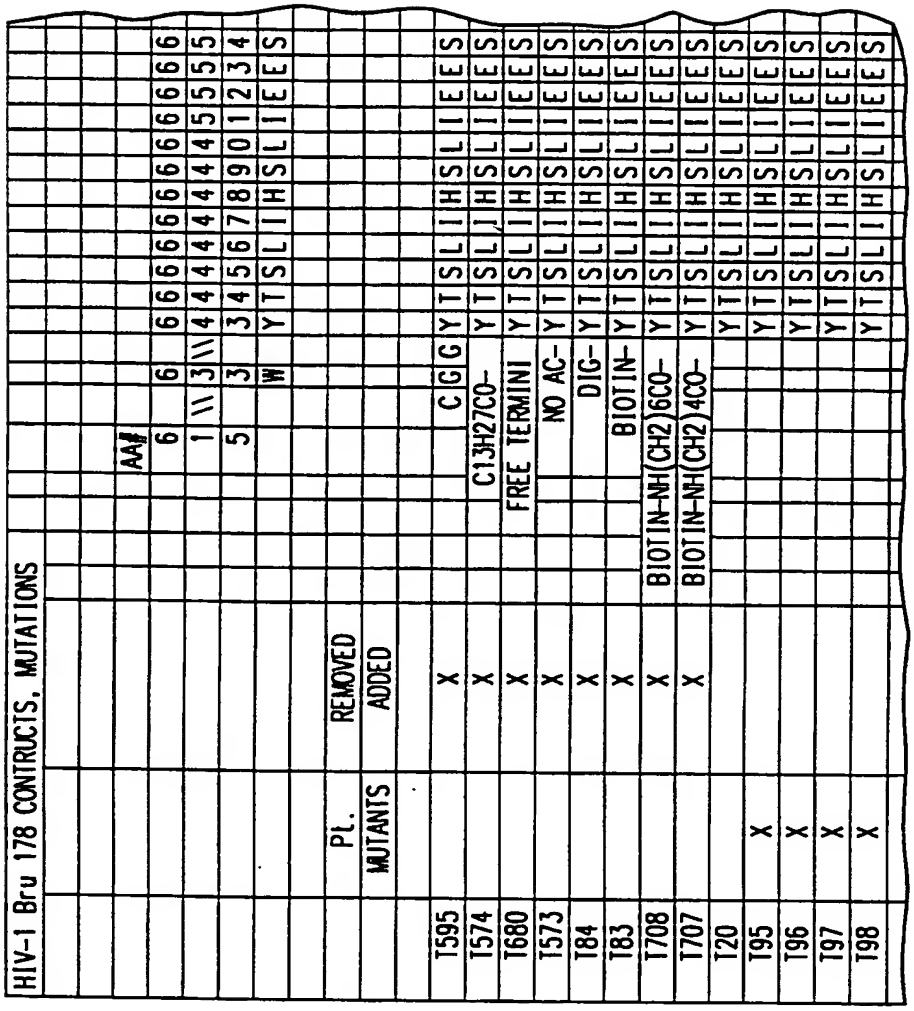

T220
T221
T234
T235
T570
T381
T382
T677
T376
T589
T377
T590
T378
T591
T270
T271
T272
T273
T608
T609
T610
T611
T612
T222
T223
T60/T224
T225
T226
T227
CYCLIZED-
CYCLIZED-
CYCLIZED-

FIG.5B(III)


HIV-1 Bru 178 CONTRUCTS, MUTATIONS
AA#
Pt. MUTANTS
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ADDED
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T574
T680
T573
T84
T83
T708
T707
T20
T95
T96
T97
T98
C13H27CO–
FREE TERMINI
NO AC–
DIG–
BIOTIN–
BIOTIN–NH(CH2)6CO–
BIOTIN–NH(CH2)4CO–

FIG.5C(I)

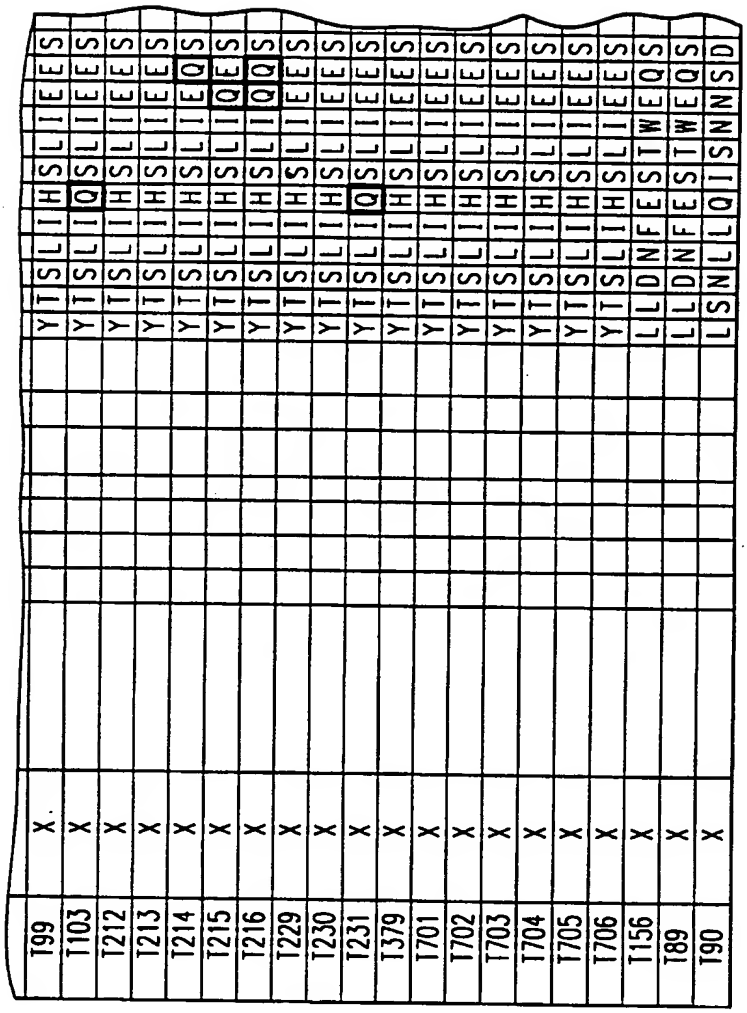
T99
T103
T212
T213
T214
T215
T216
T229
T230
T231
T379
T701
T702
T703
T704
T705
T706
T156
T89
T90

FIG.5C(III)

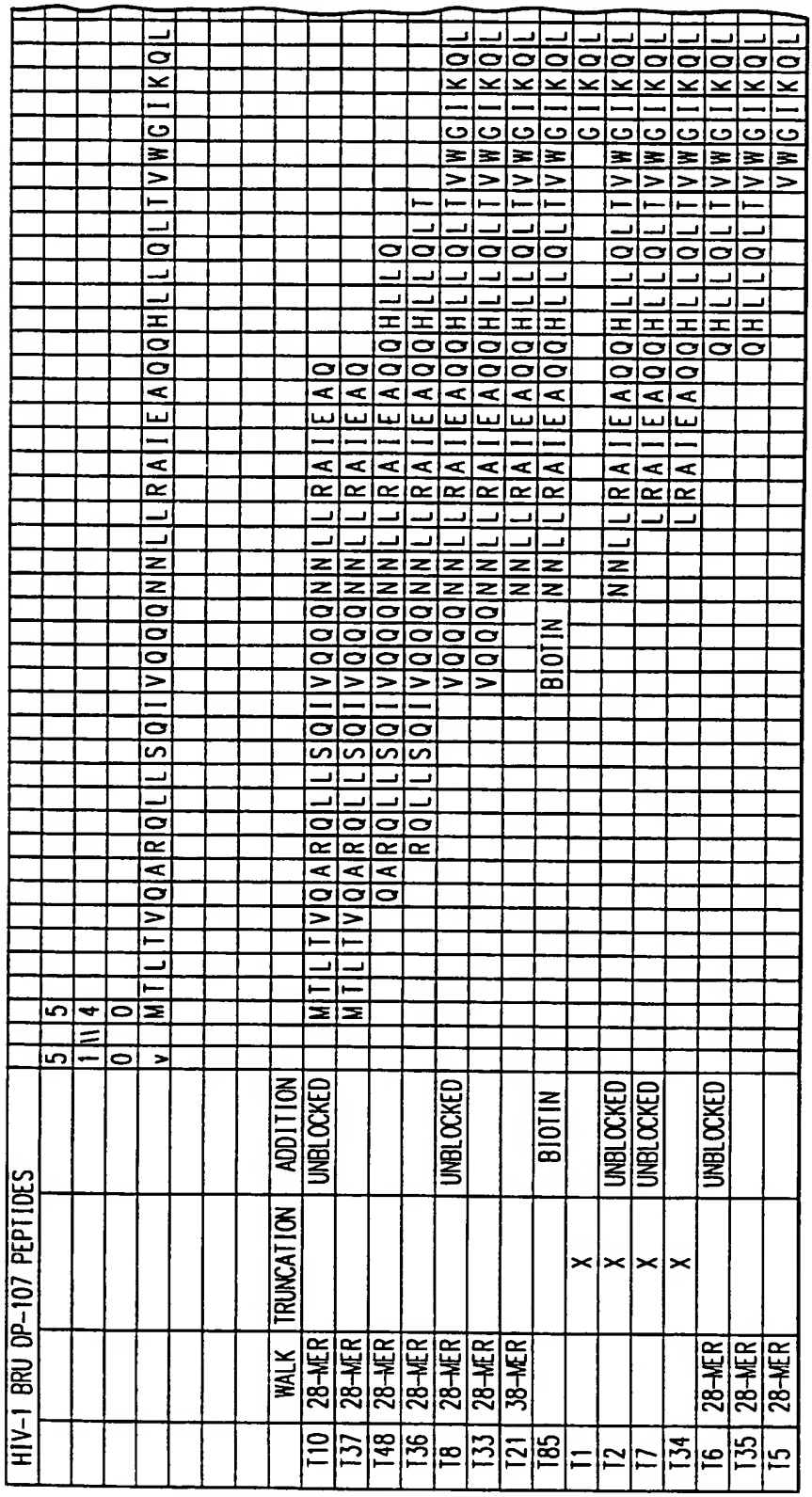
HIV-1 BRU OP-107 PEPTIDES
WALK
TRUNCATION
ADDITION
T10 28-MER UNBLOCKED
T37 28-MER
T48 28-MER
T36 28-MER
T8 28-MER UNBLOCKED
T33 28-MER
T21 38-MER
T85 BIOTIN
T1 X
T2 X UNBLOCKED
T7 X UNBLOCKED
T34 X
T6 28-MER UNBLOCKED
T35 28-MER
T5 28-MER

FIG.6(I)

SIMIAN IMMUNODEFICIENCY VIRUS MM251		
DP178-LIKE		
RESIDUE	245	TWQEWERKVDFLEENITALLEEAQIQQEKNMYELQKLNSWDVFGNWF
T- 390	245	TWQEWERKVDFLEENITALLEEAQIQQEKNMYELQ
T- 391	246	WQEWERKVDFLEENITALLEEAQIQQEKNMYELQK
T- 392	247	QEWERKVDFLEENITALLEEAQIQQEKNMYELQKL
T- 393	248	EWERKVDFLEENITALLEEAQIQQEKNMYELQKLN
T- 394	249	WERKVDFLEENITALLEEAQIQQEKNMYELQKLNS
T- 395	250	ERKVDFLEENITALLEEAQIQQEKNMYELQKLNSW
T- 396	251	RKVDFLEENITALLEEAQIQQEKNMYELQKLNSWD
T- 397	252	KVDFLEENITALLEEAQIQQEKNMYELQKLNSWDV
T- 398	253	VDFLEENITALLEEAQIQQEKNMYELQKLNSWDVF
T- 399	254	DFLEENITALLEEAQIQQEKNMYELQKLNSWDVFG
T- 400	255	FLEENITALLEEAQIQQEKNMYELQKLNSWDVFGN

FIG.7(I)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09499

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 38/00, 38/02; C07K 1/00, 5/00, 7/00, 17/00
US CL :514/12; 530/300, 324, 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/12; 530/300, 324, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, BIOSIS, CAS ONLINE, MEDLINE.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,141,867 (IVANOFF et al.) 25 August 1992, see entire document.	1-33
Y	US, A, 5,077,280 (SOMMADOSSI et al.) 31 December 1991, see entire document.	1-33
Y	US, A, 4,950,652 (CARTER) 21 August 1990, see entire document.	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
19 AUGUST 1996

Date of mailing of the international search report
02 OCT 1996

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